

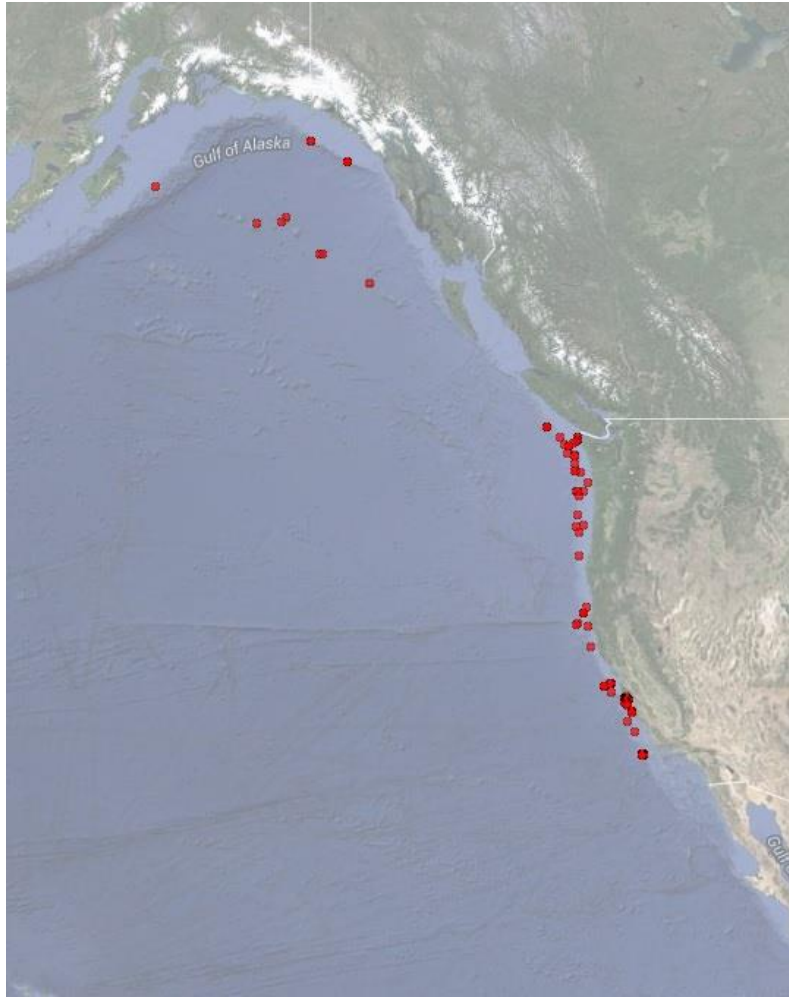
**NOAA**  
**FISHERIES**

NWFSC

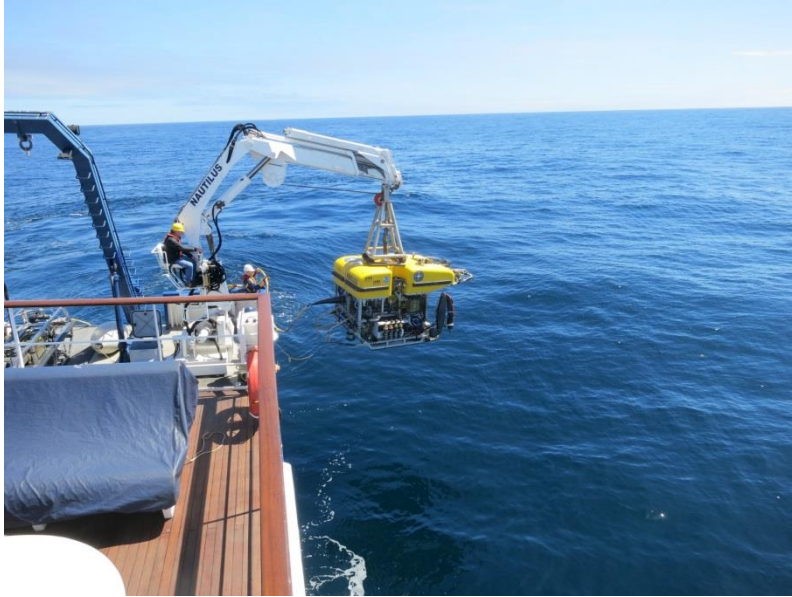
# From population structure to eDNA: Next-generation sequencing technology opens a window into the biology of deep-sea corals

Meredith V. Everett, Linda K. Park, Ewann A. Berntson, Cheryl L. Morrison, Robert P. Stone, Anna E. Elz, Curt E. Whitmire, Aimee A. Keller, and M. Elizabeth Clarke, and Kathryn Kegel

Understanding species distribution, population connectivity, and human impacts is critical to management of deep-sea coral populations. A key factor in obtaining this information is sample collection.

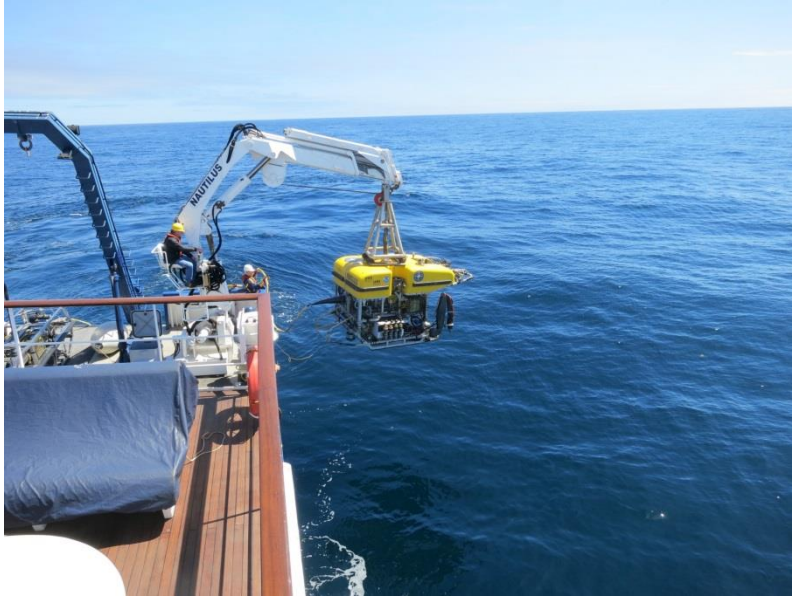


# Obtaining samples remains a challenge for study

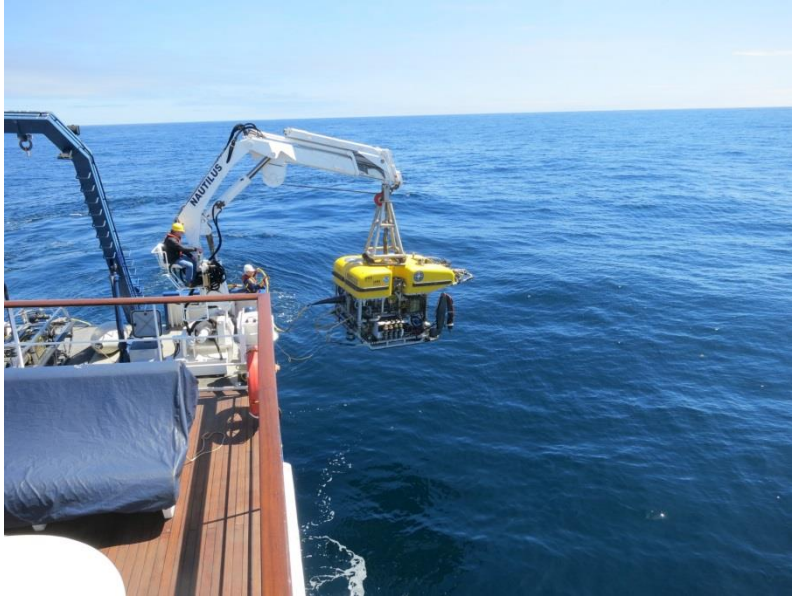




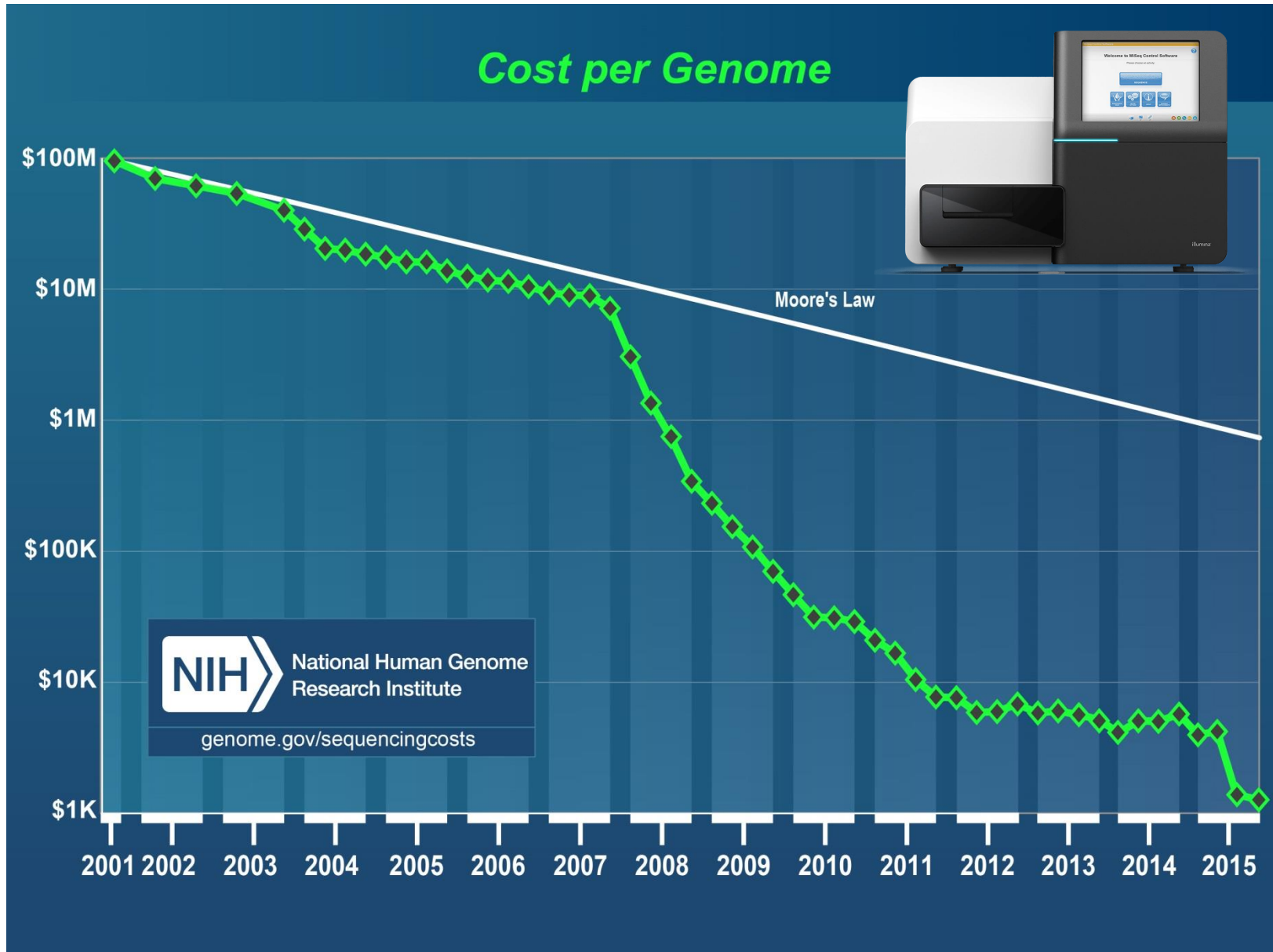
# Obtaining samples remains a challenge for study



# Obtaining samples remains a challenge for study



# Next generation sequencing can help overcome limitations of sampling





# Two applications of next-generation sequencing in deep-sea corals: RAD-tags and eDNA

Image Credit: Ocean Exploration Trust



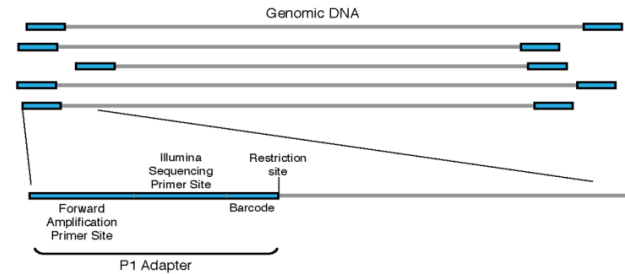
**NOAA FISHERIES**

U.S. Department of Commerce | National Oceanic and Atmospheric Administration | NOAA Fisheries | Northwest Fisheries Science Center

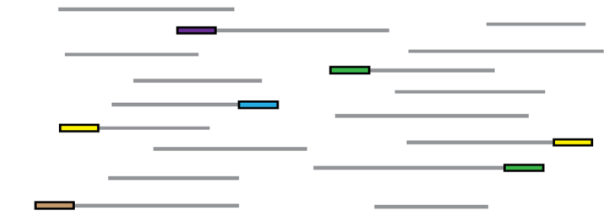
# RAD-tag sequencing

- Uses Illumina sequencing methods to sequence directly adjacent to restriction site

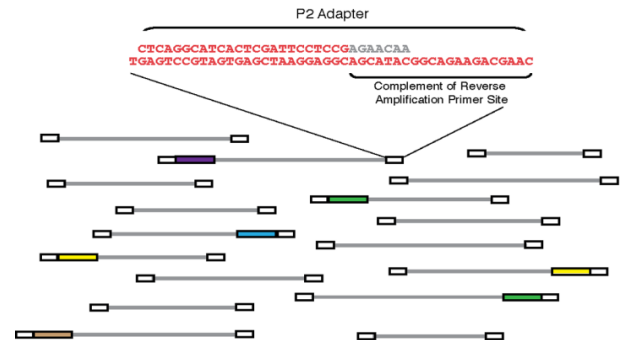
## A *Ligate P1 Adapter to digested genomic DNA*



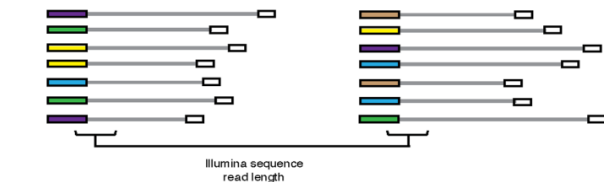
## B *Pool barcoded samples and shear*



## C *Ligate P2 Adapter to sheared fragments*



## D *Selectively amplify RAD tags*

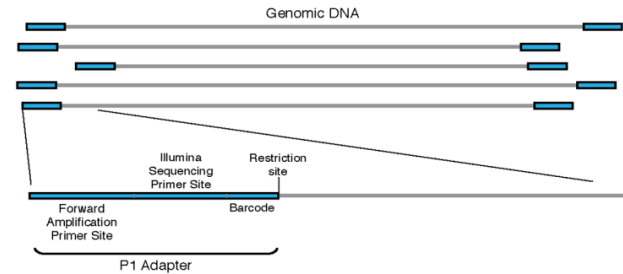




# RAD-tag sequencing

- Uses Illumina sequencing methods to sequence directly adjacent to restriction site
- Samples are barcoded allowing individual genotyping

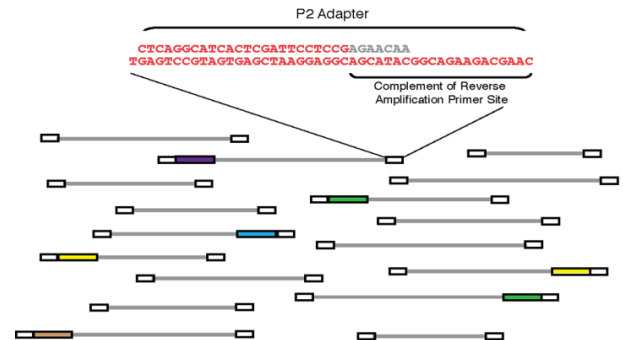
## A *Ligate P1 Adapter to digested genomic DNA*



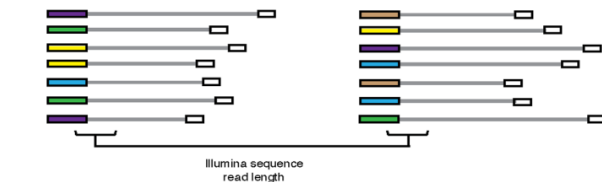
## B *Pool barcoded samples and shear*



## C *Ligate P2 Adapter to sheared fragments*



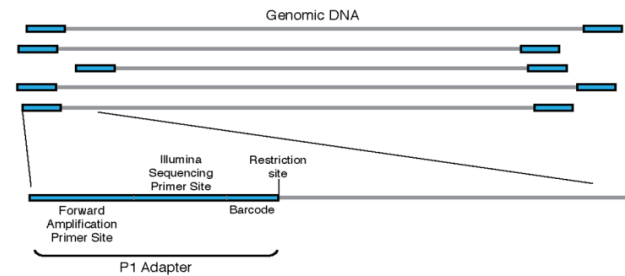
## D *Selectively amplify RAD tags*



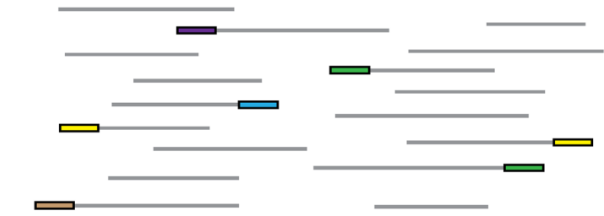
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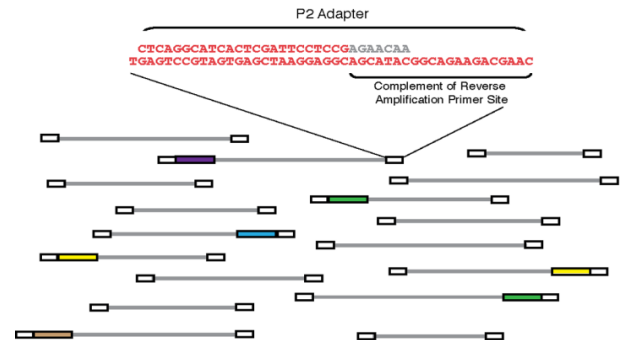
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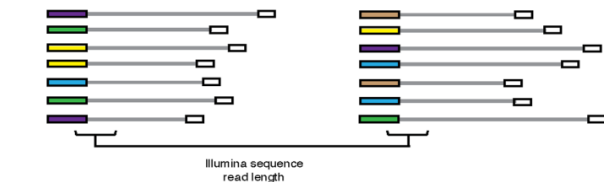
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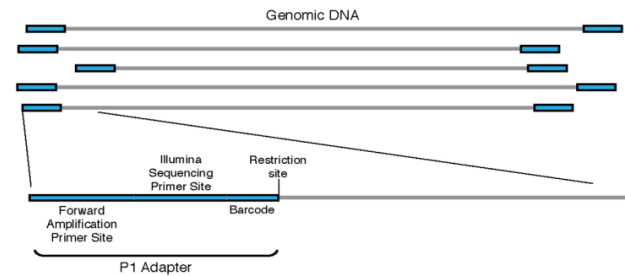
## D *Selectively amplify RAD tags*



# RAD-tag sequencing

- Uses Illumina sequencing methods to sequence directly adjacent to restriction site
- Samples are barcoded allowing individual genotyping

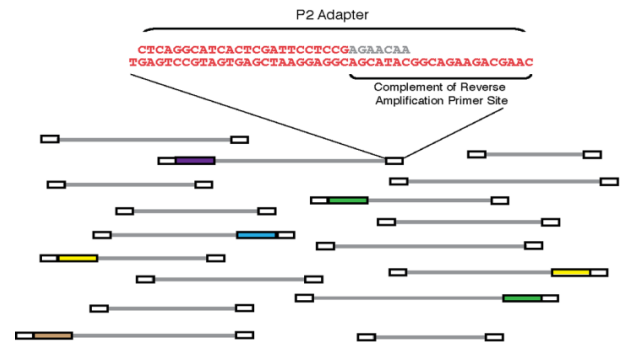
## A *Ligate P1 Adapter to digested genomic DNA*



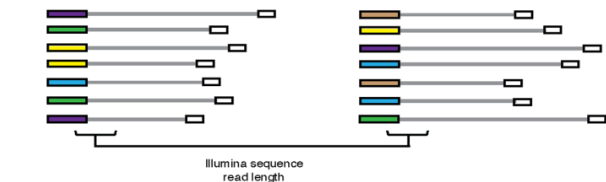
## B *Pool barcoded samples and shear*



## C *Ligate P2 Adapter to sheared fragments*



## D *Selectively amplify RAD tags*

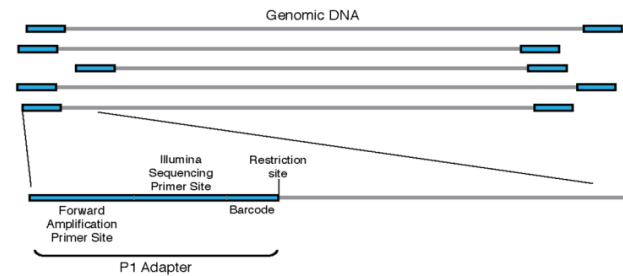




# RAD-tag sequencing

- Uses Illumina sequencing methods to sequence directly adjacent to restriction site
- Samples are barcoded allowing individual genotyping
- Allows rapid identification and genotyping of thousands of novel SNPs in non-model species

## A *Ligate P1 Adapter to digested genomic DNA*



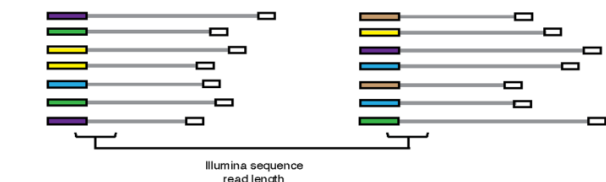
## B *Pool barcoded samples and shear*



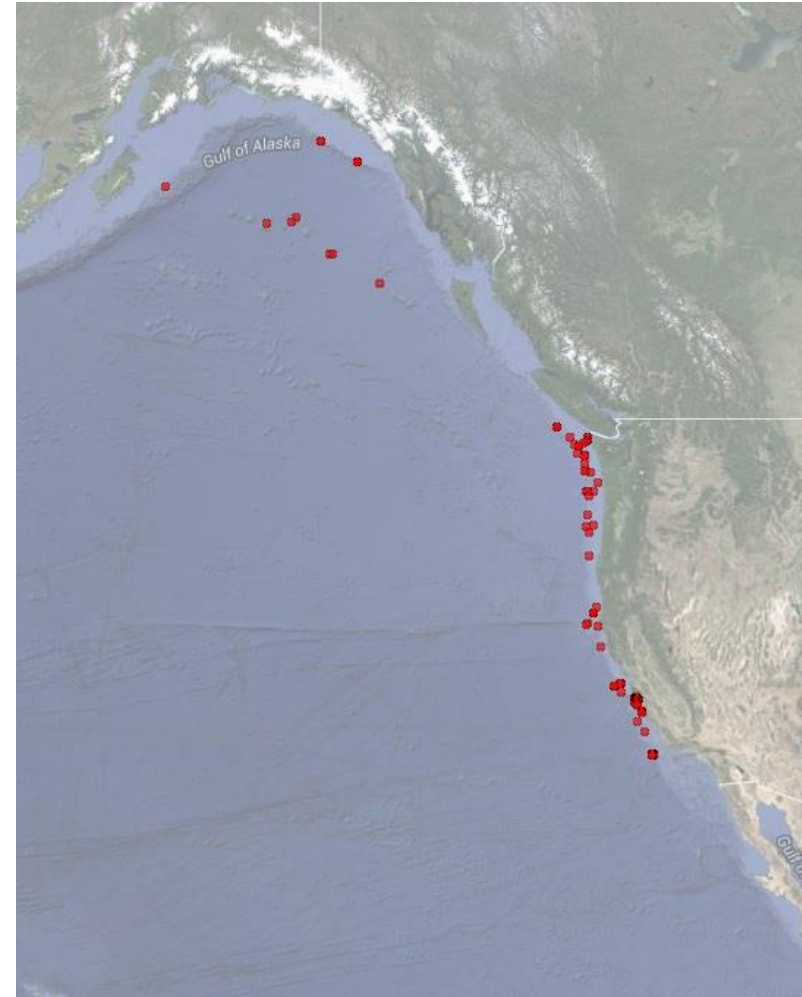
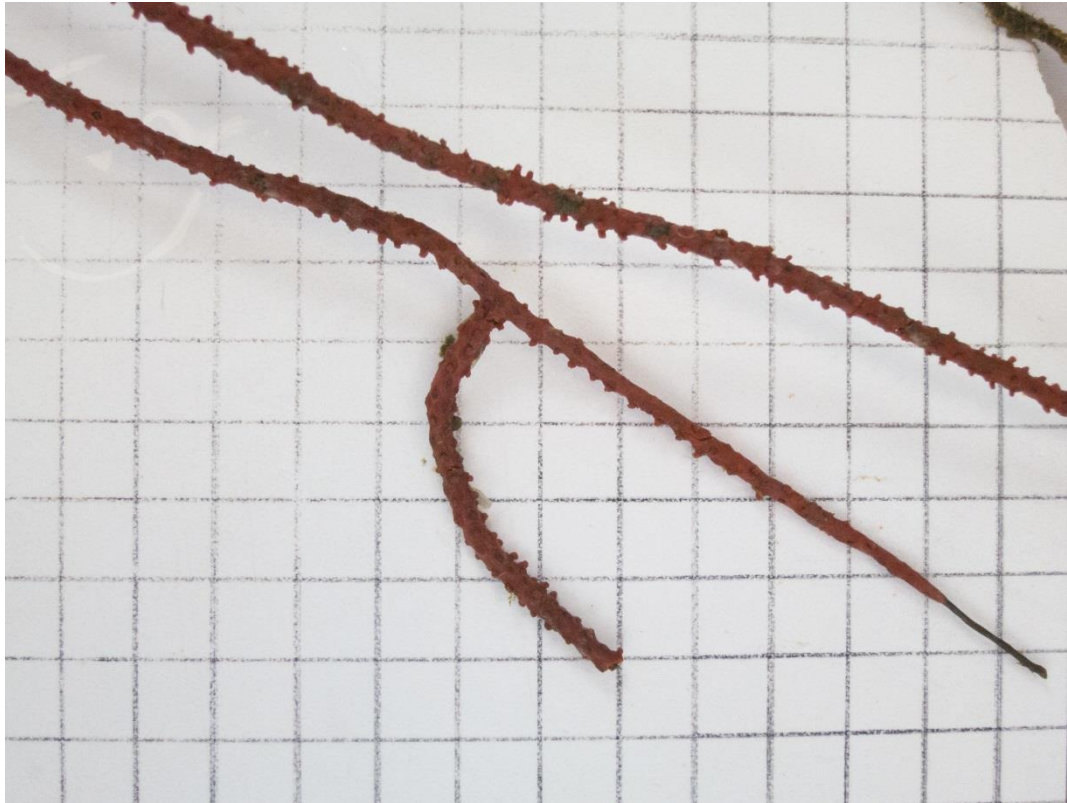
## C *Ligate P2 Adapter to sheared fragments*



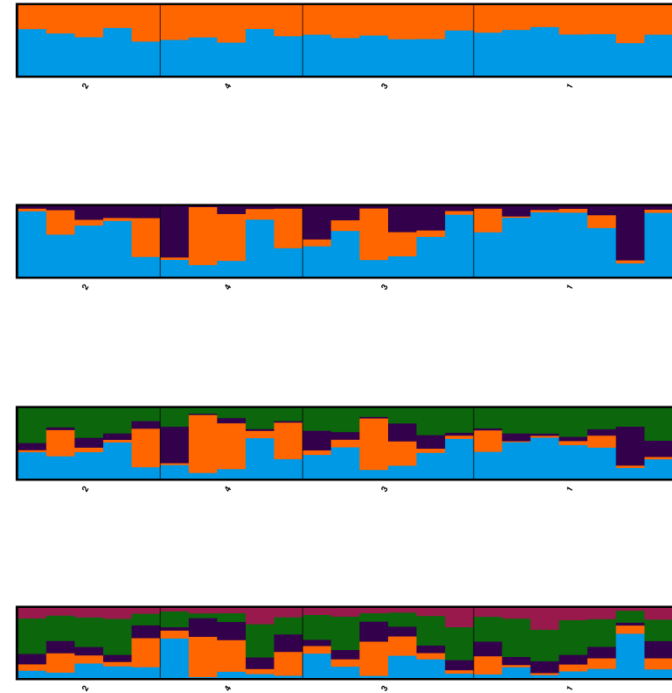
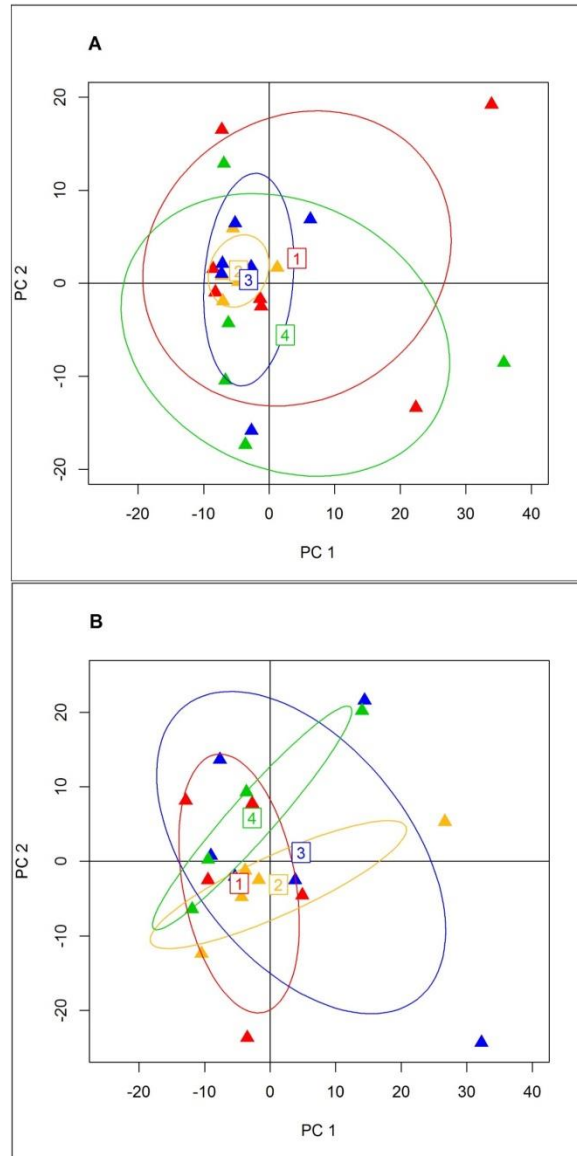
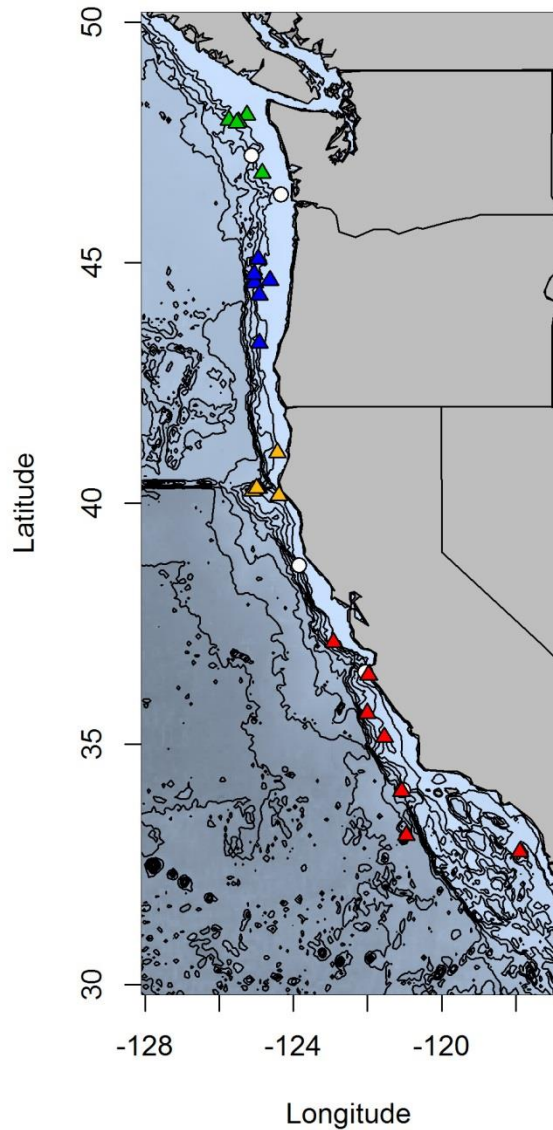
## D *Selectively amplify RAD tags*



# *Swiftia simplex*



# Swiftia simplex



$$F_{ST} = 0.0056 \text{ (} p=0.30 \text{)}$$

$$\text{IBD (} p=0.099 \text{)}$$

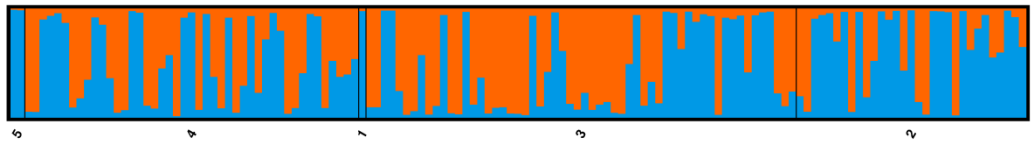
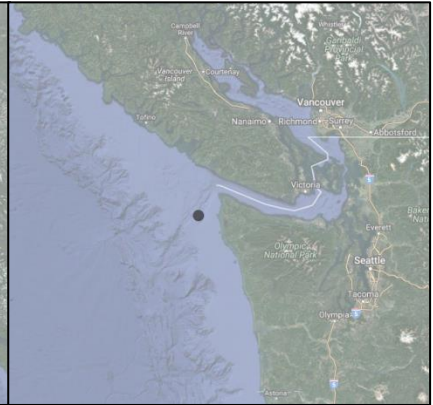
No significant pairwise  $F_{ST}$



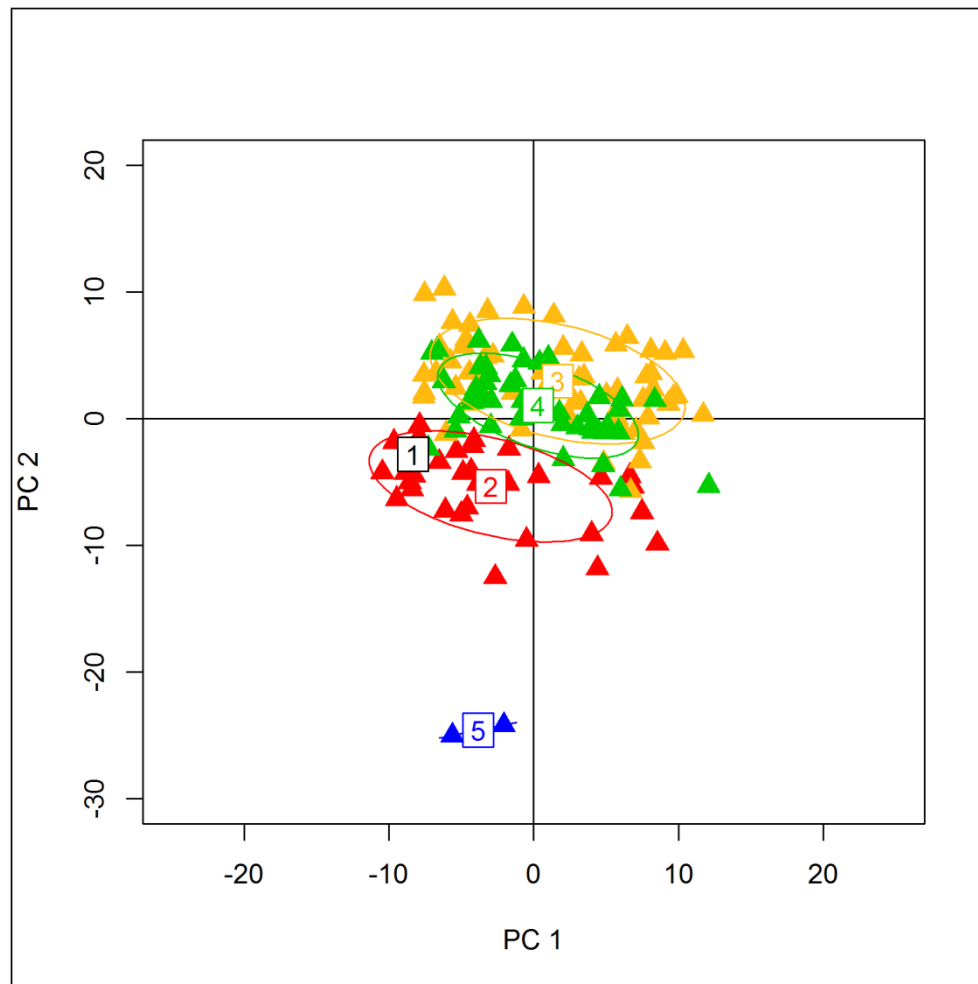
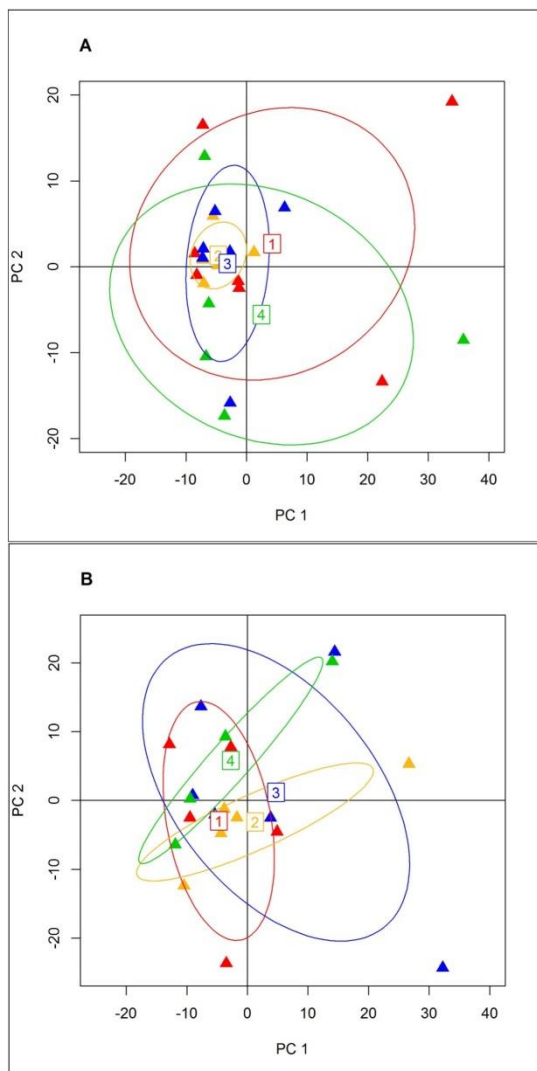
# *Primnoa pacifica*



# *Primnoa pacifica*

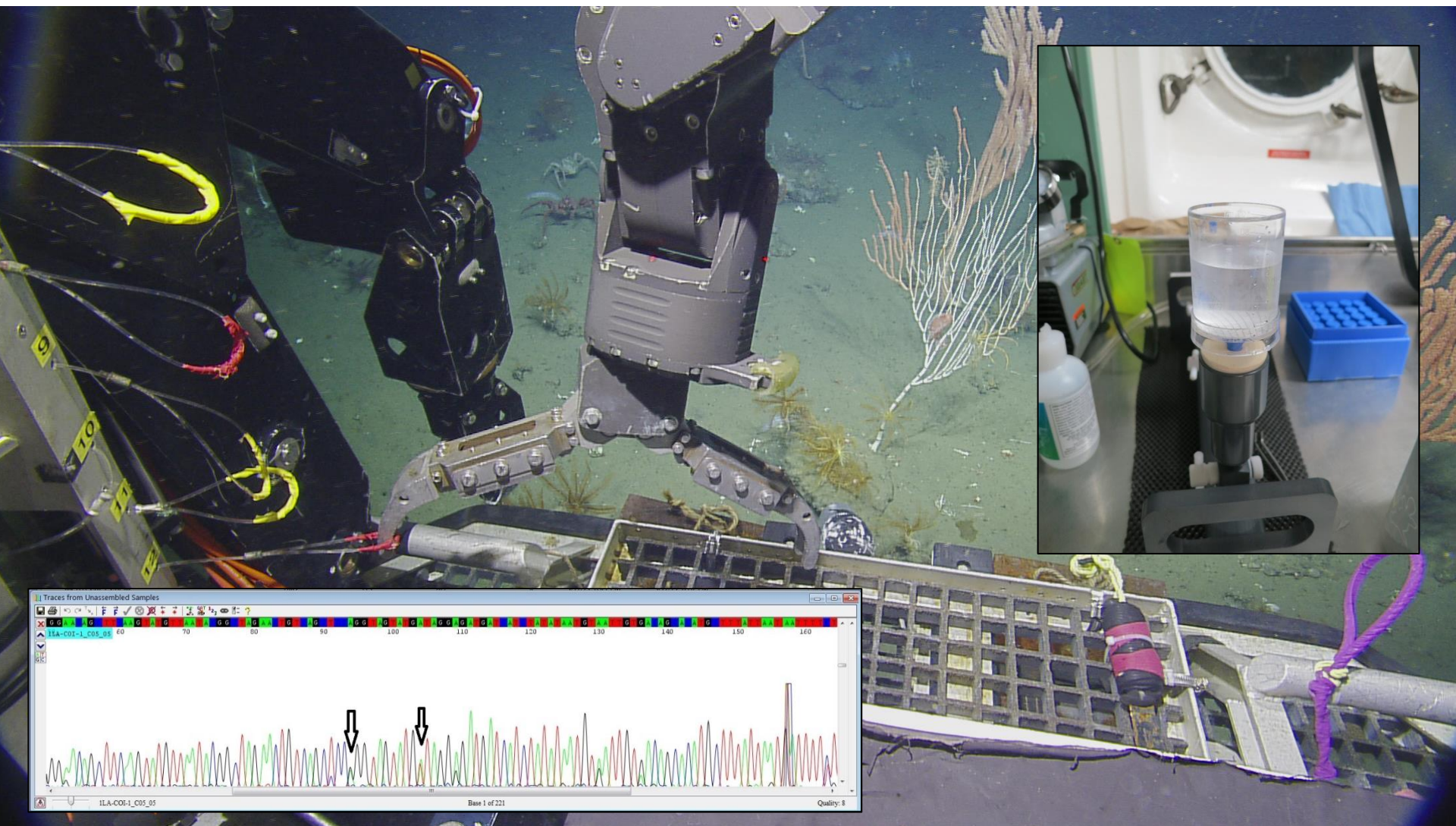


# Must look at each species of interest





# Increasing Sample Opportunity: eDNA



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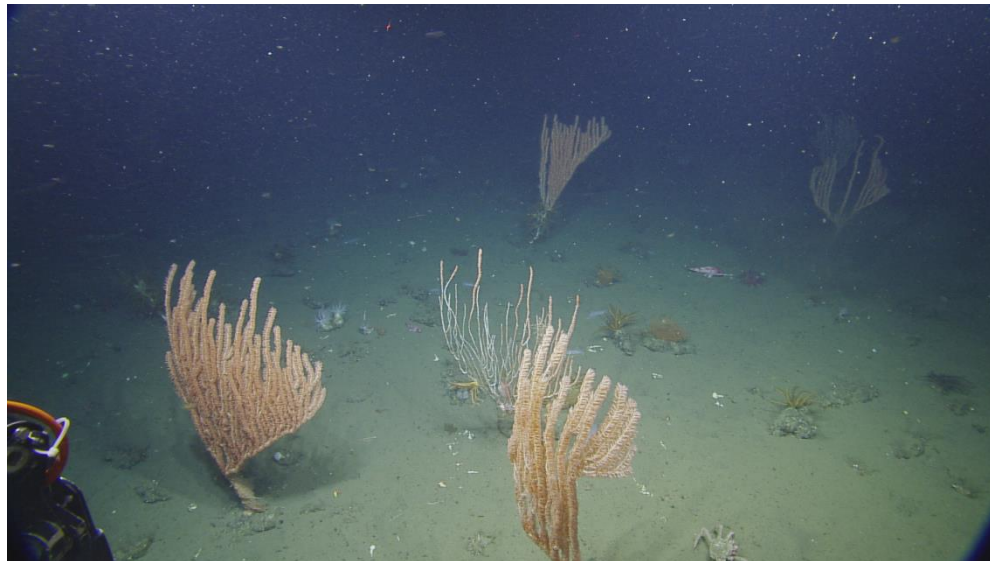
Image Credit: Ocean Exploration Trust



# Why use eDNA: Species identification

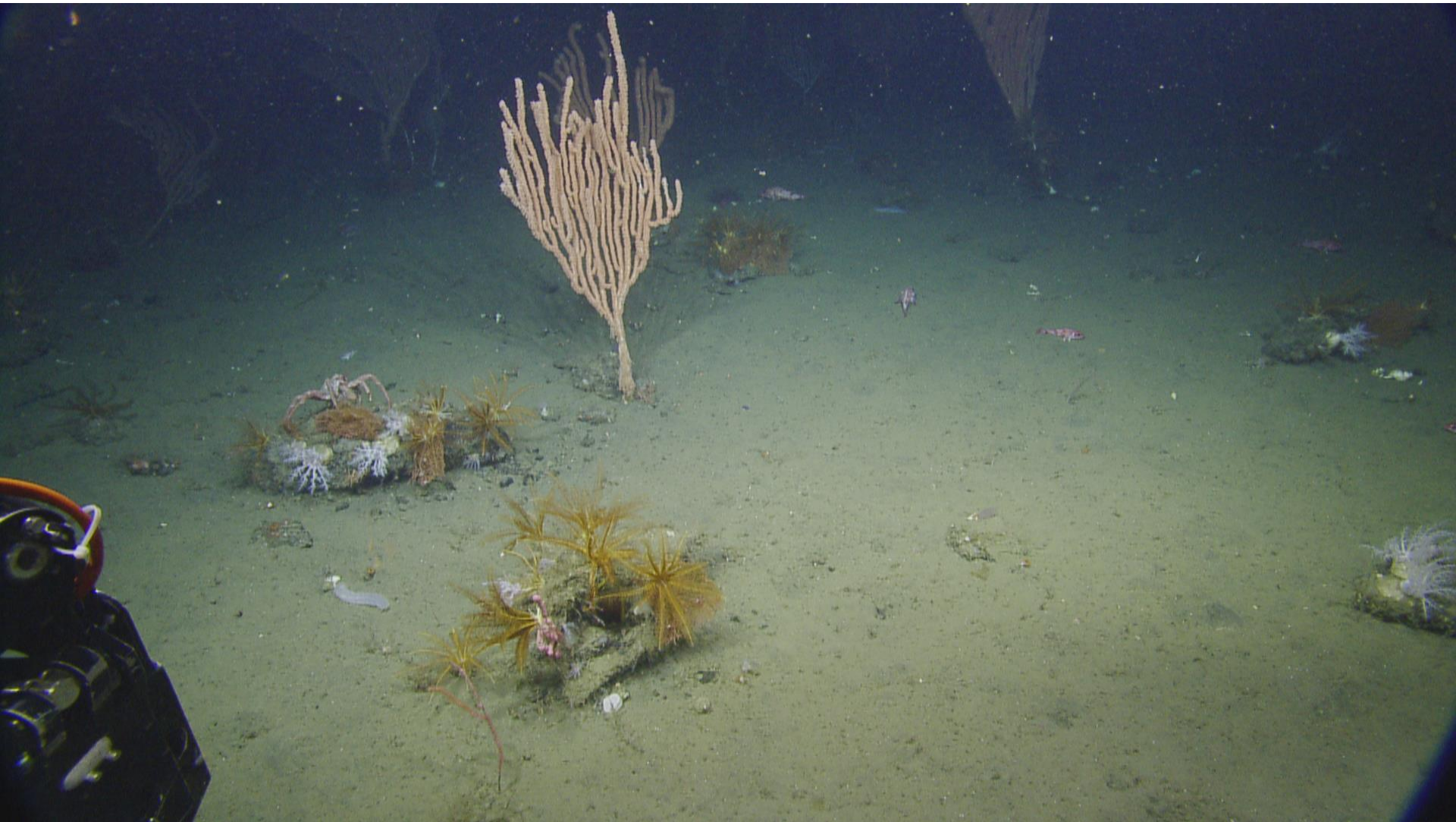


# Why use eDNA: Species identification



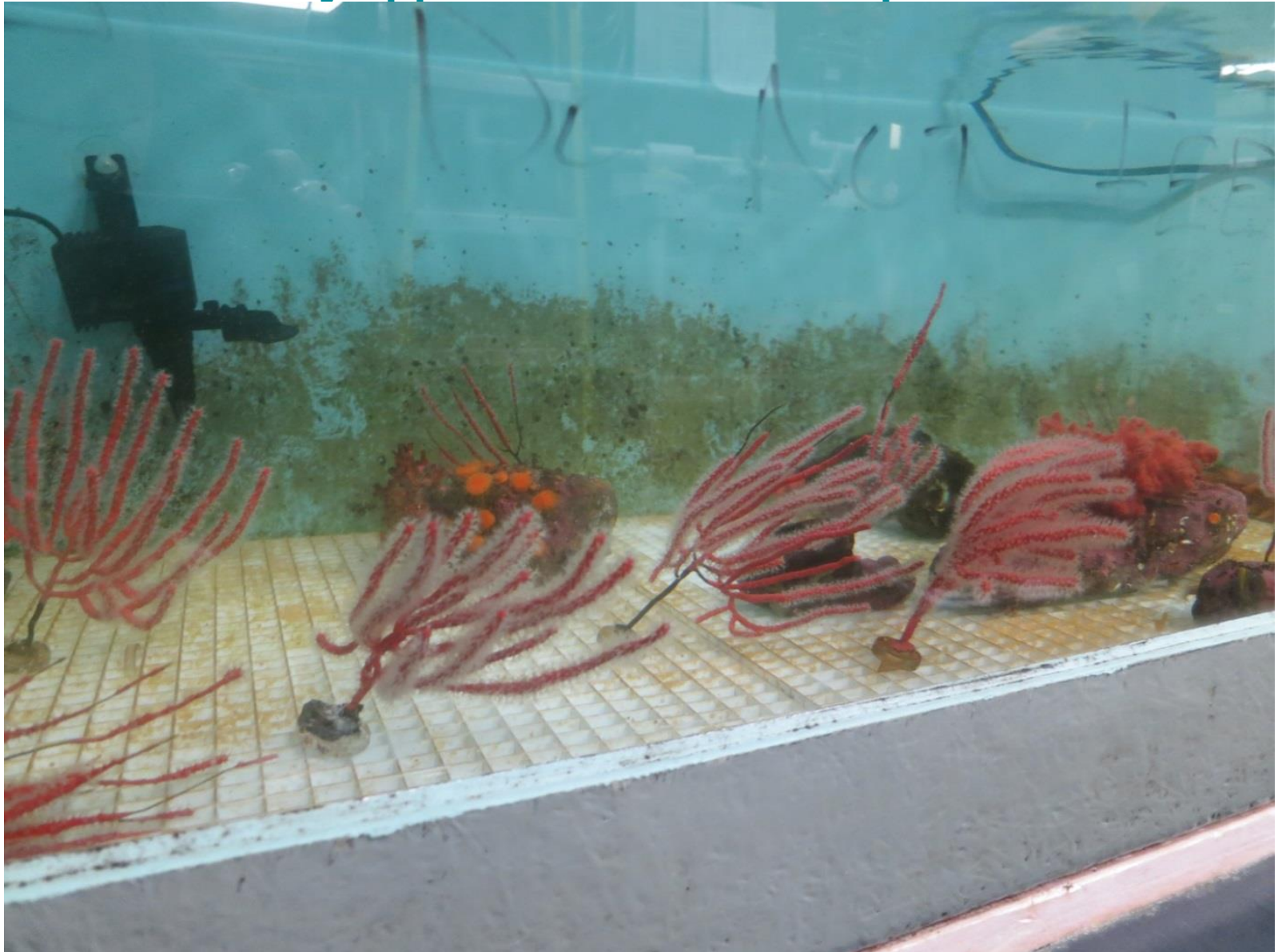


# Increasing Sample Opportunity: eDNA





## Early applications: Seattle Aquarium

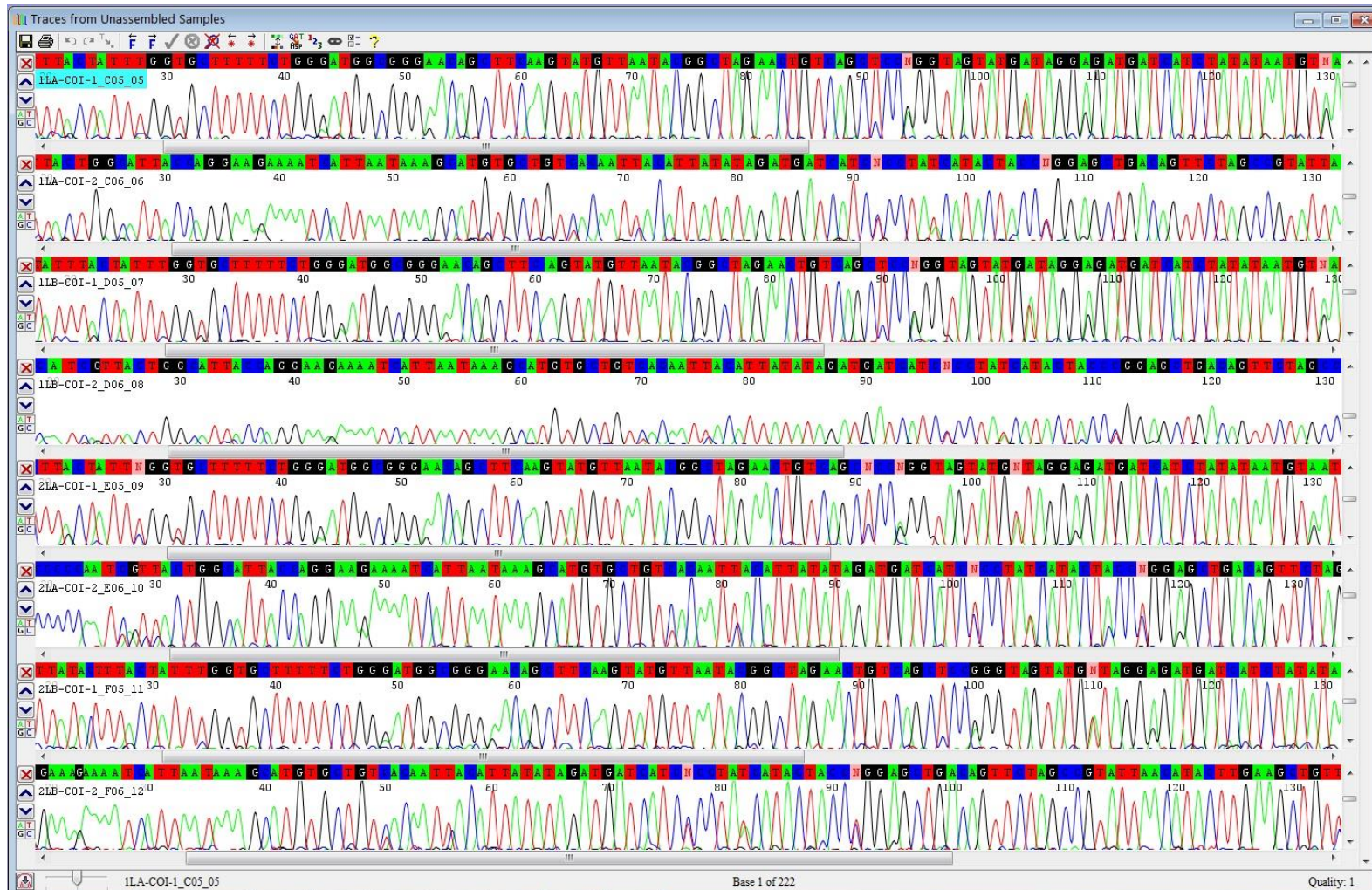


## Early applications: Seattle Aquarium



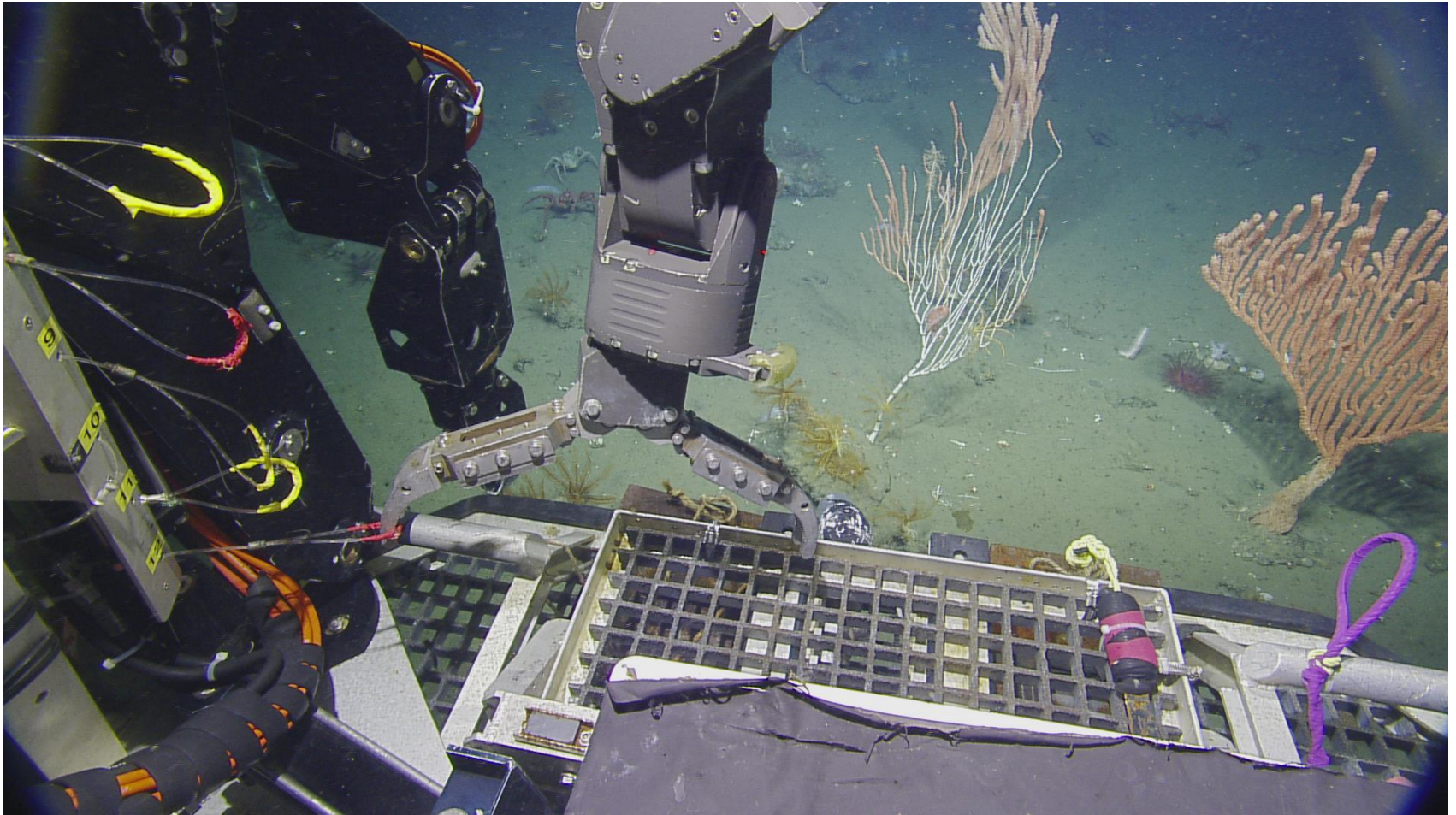


# Early applications: Seattle Aquarium





## Testing in the field: EV Nautilus





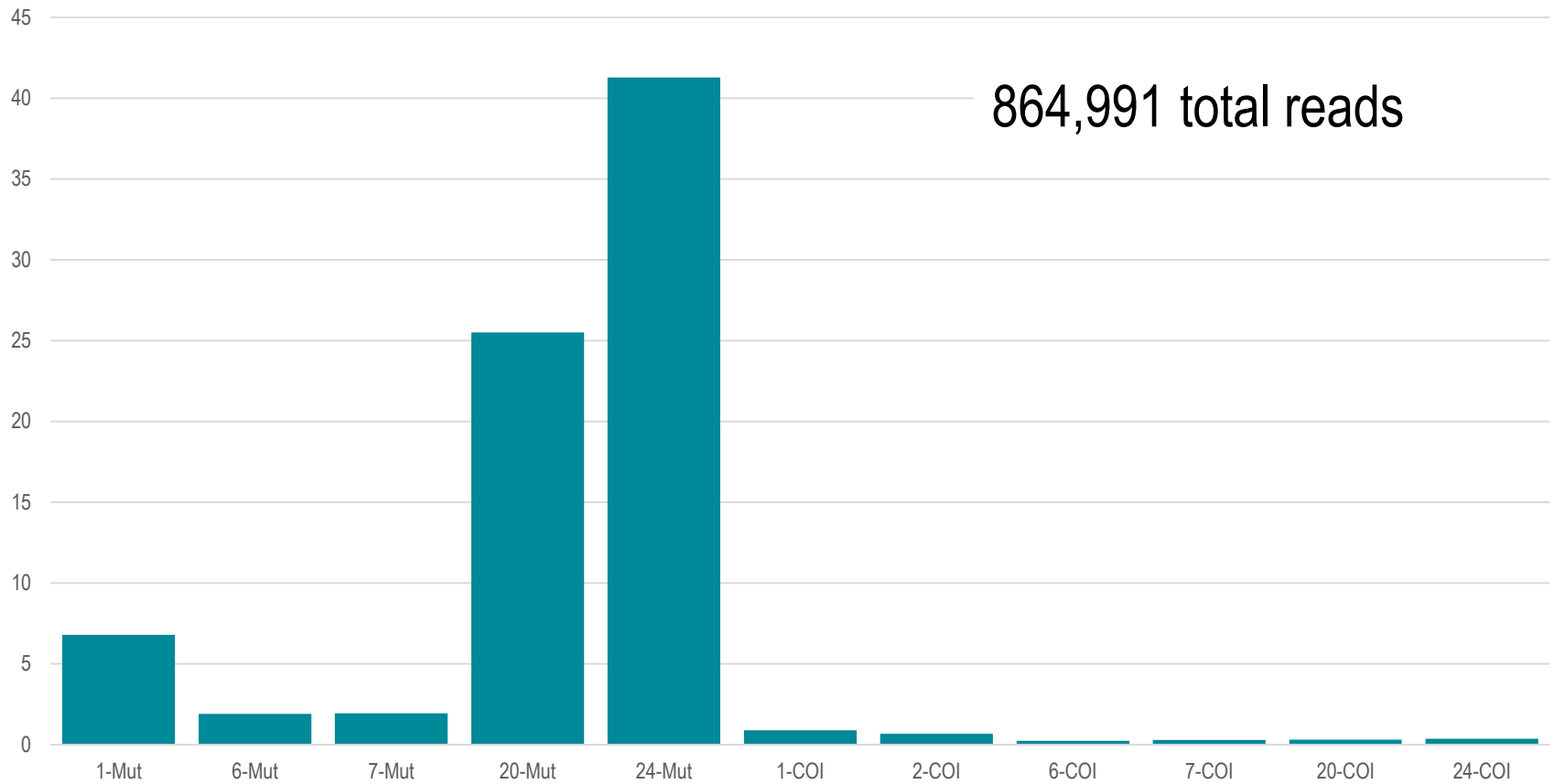


- More than 50 samples successfully extracted
- Designed Octocoral primers for two loci, should amplify all families
- Successful initial PCR
- MiSeq test run on 5 samples

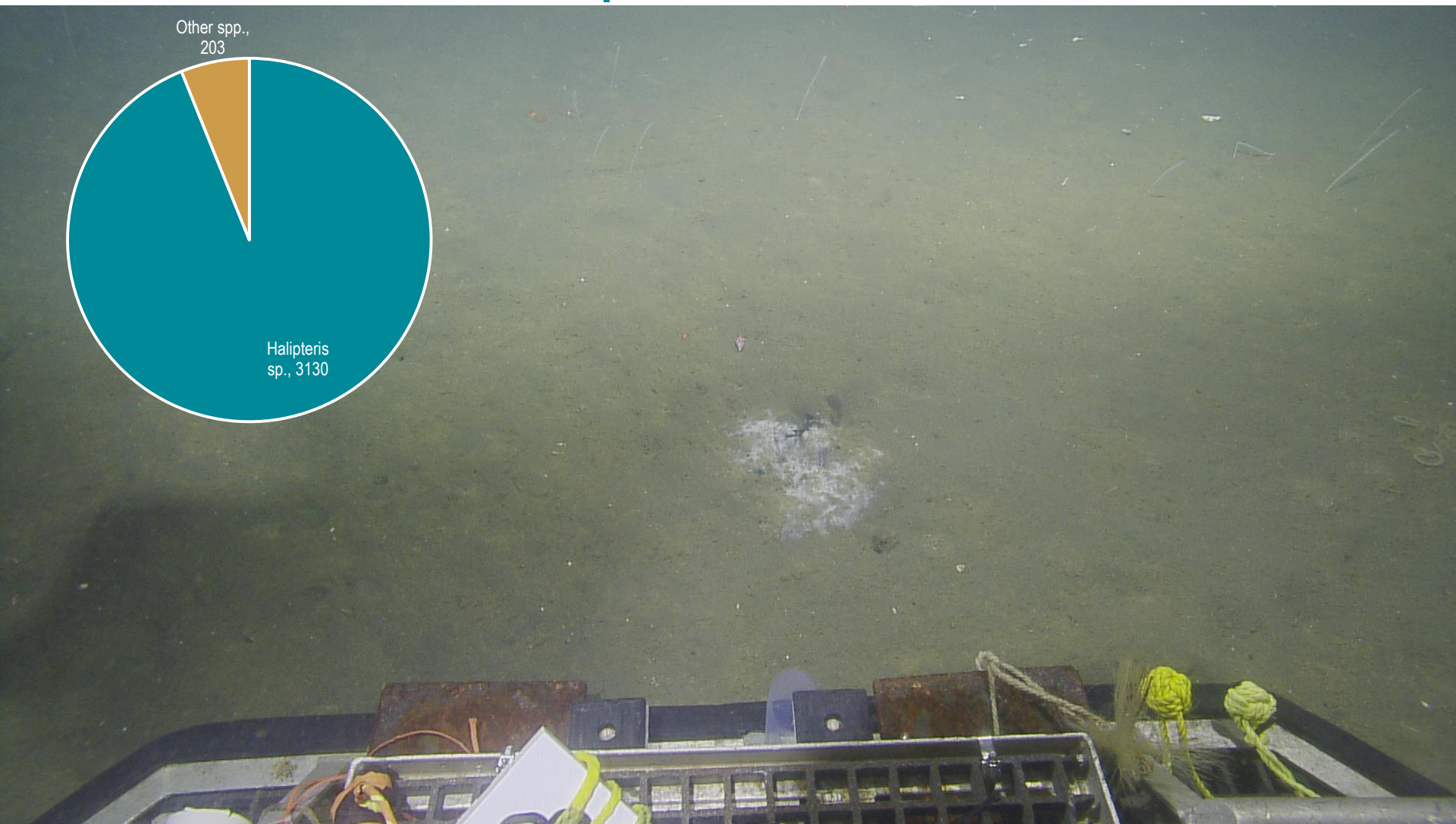
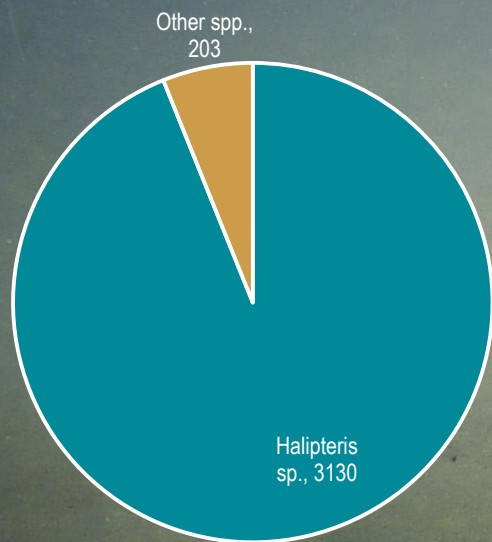


# MiSeq Results

Percent reads per index



# MiSeq Results: eDNA-1

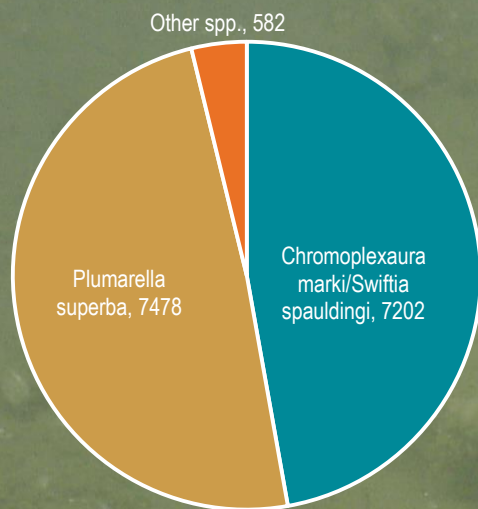


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Image Credit: Ocean Exploration Trust

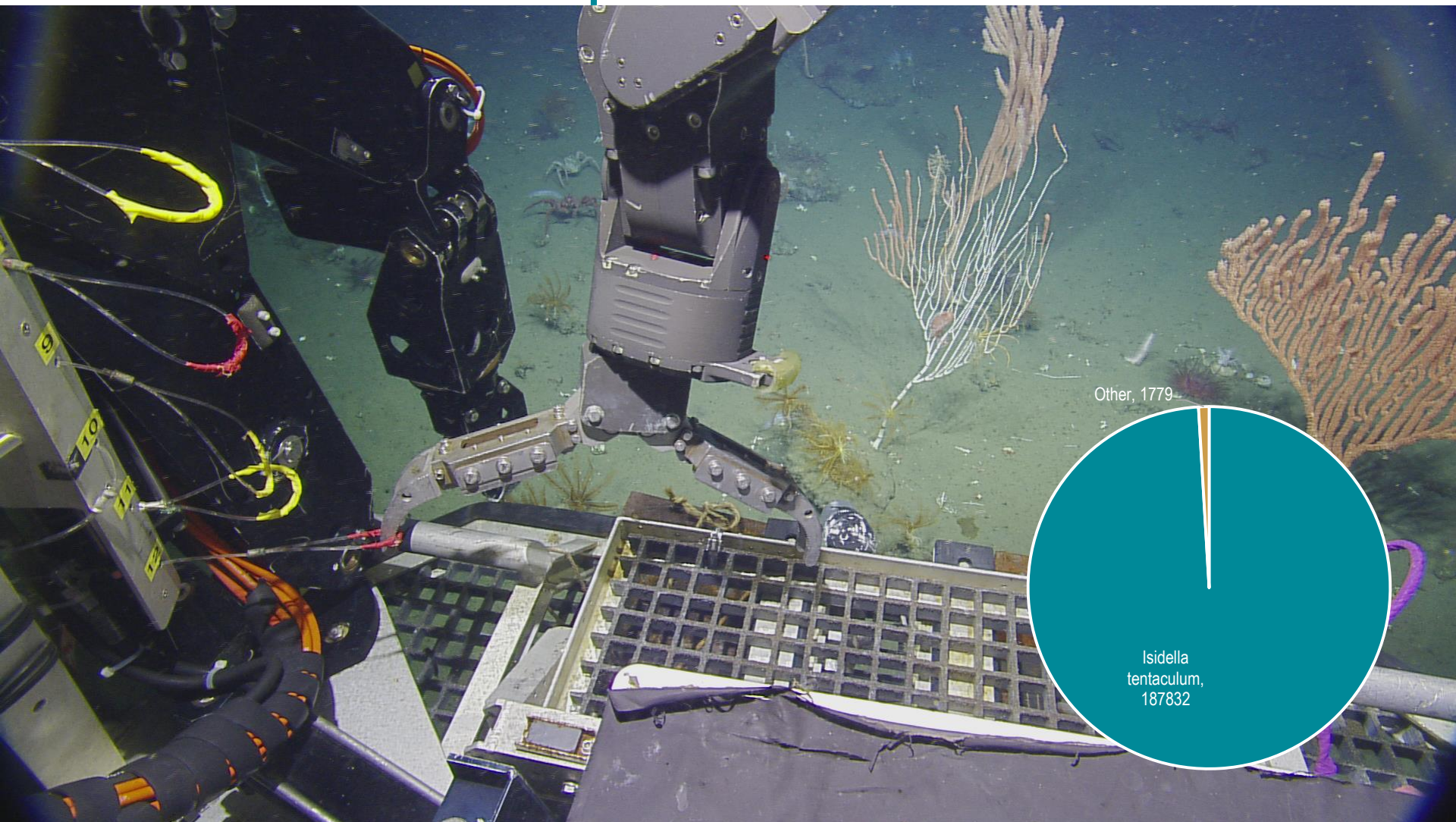


# MiSeq Results: eDNA-7





# MiSeq Results: eDNA-20

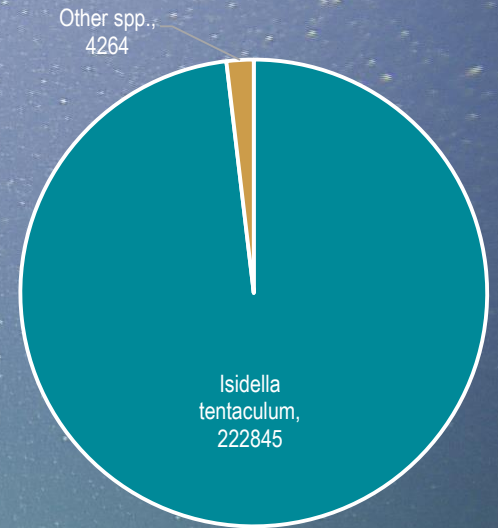
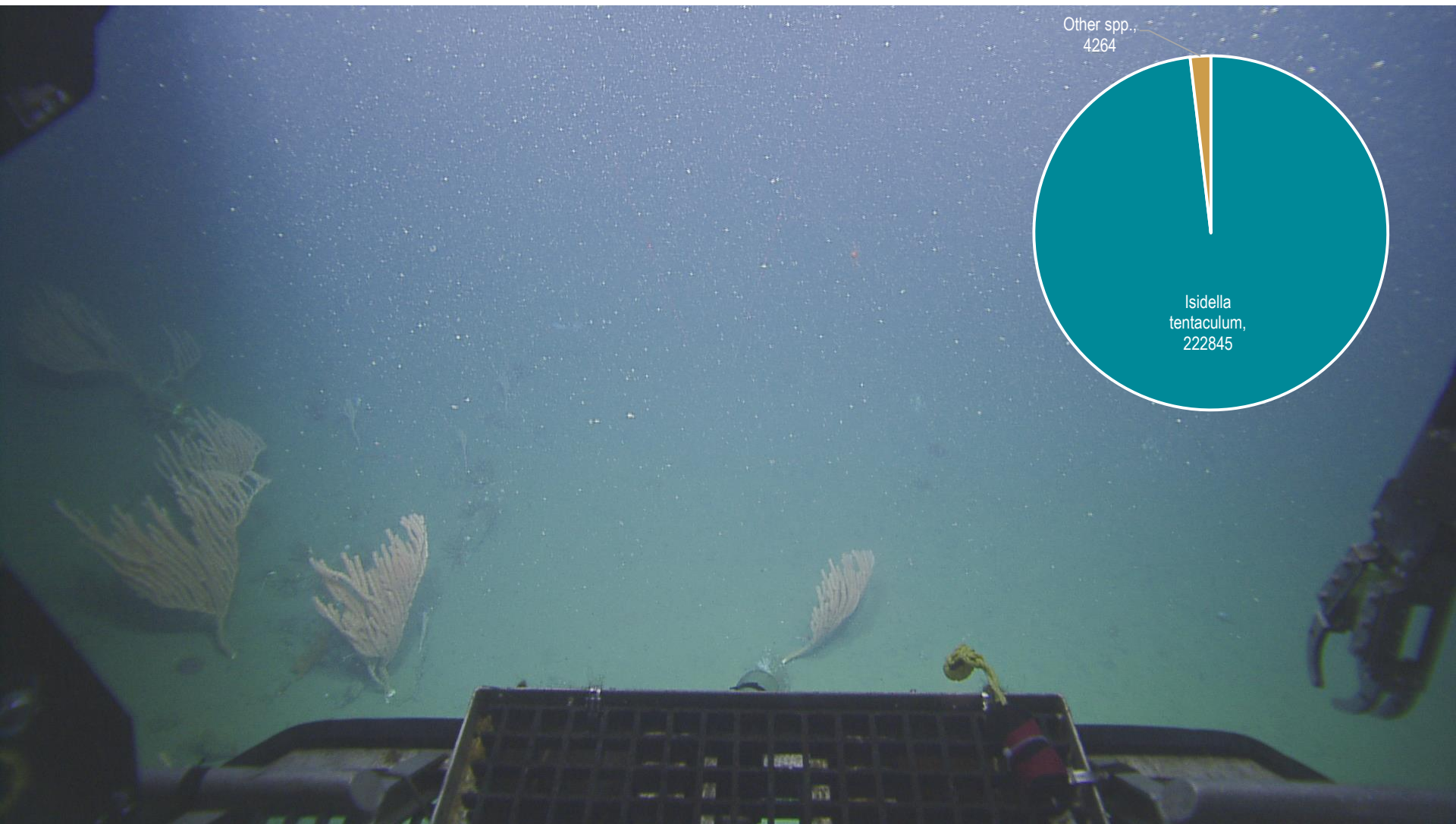


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Image Credit: Ocean Exploration Trust



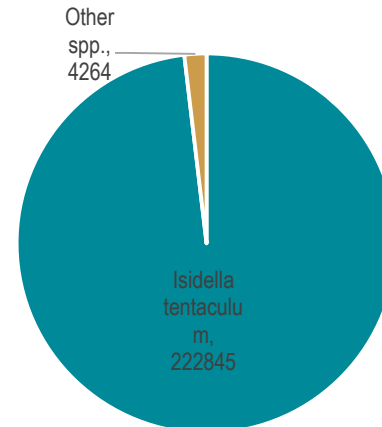
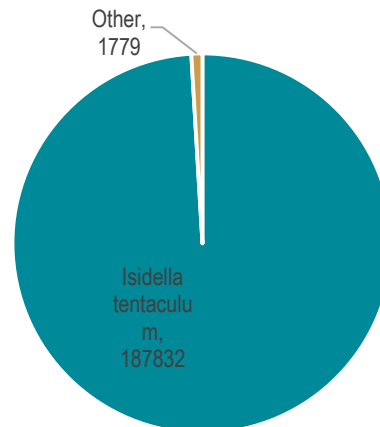
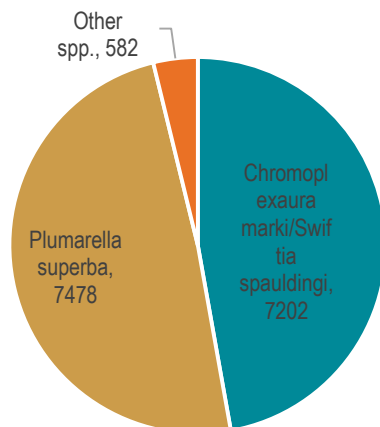
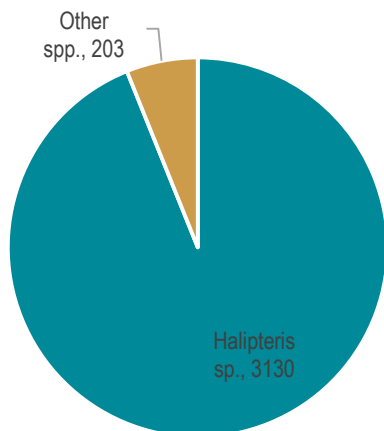
# MiSeq Results eDNA-24



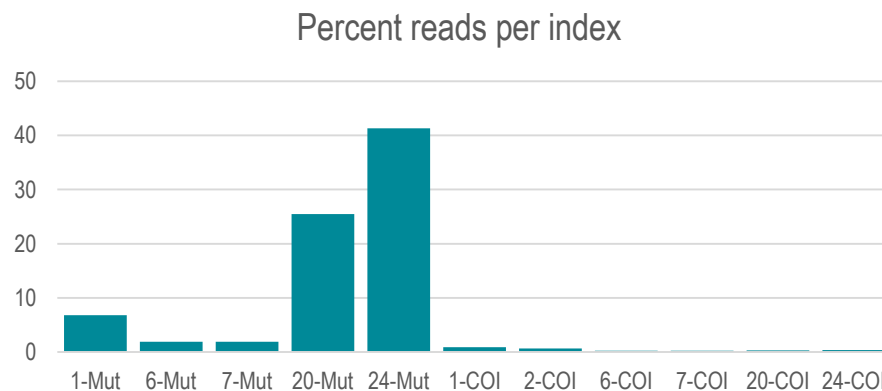
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Image Credit: Ocean Exploration Trust

## Next Steps: Optimize the method



- Test for eDNA primer bias
- Complete eDNA extraction
- Sequence full MiSeq Run to increase depth



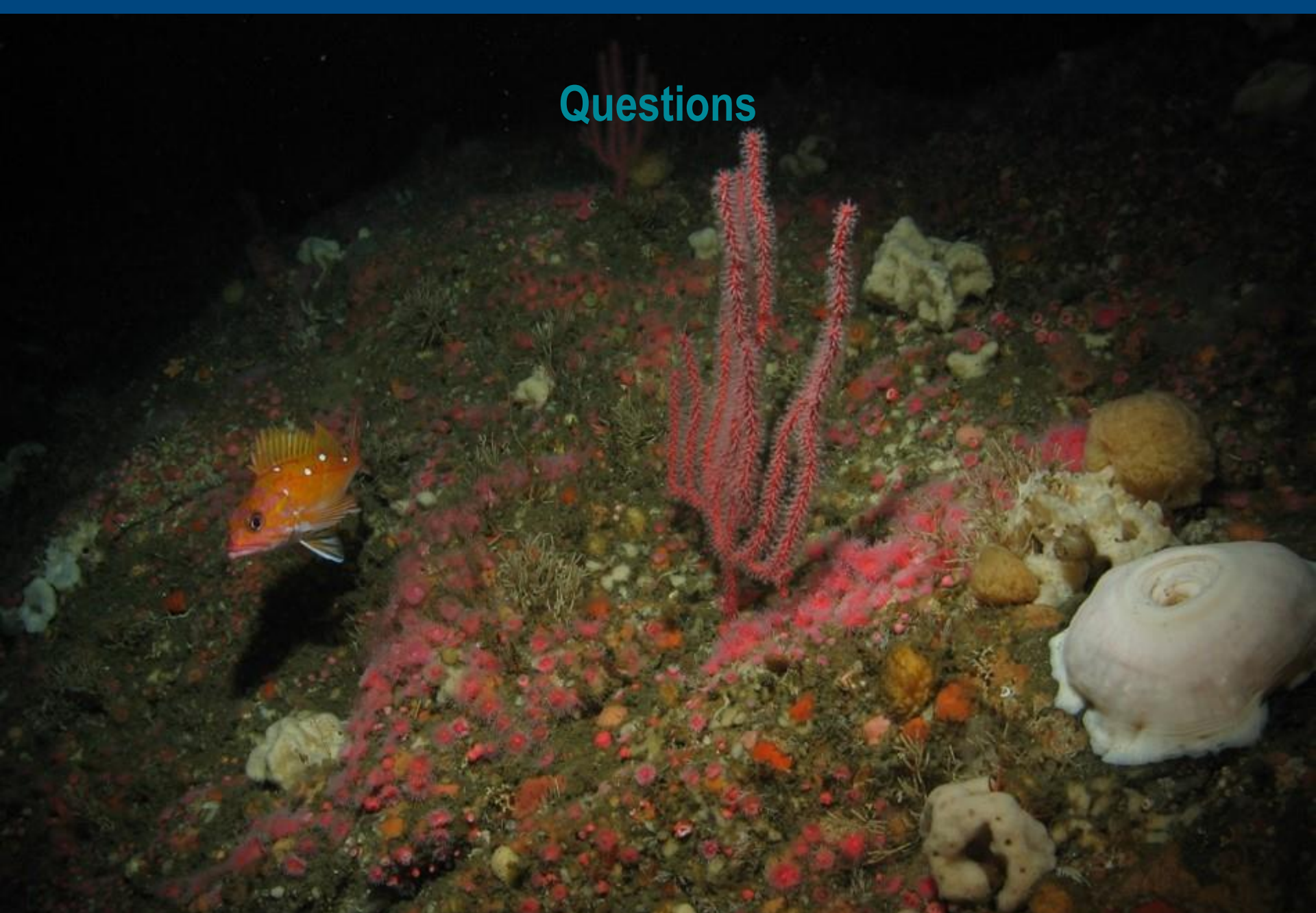
# Acknowledgements

- Co-authors: Linda K. Park, Ewann A. Berntson, Cheryl L. Morrison, Robert P. Stone Anna E. Elz, Curt E. Whitmire, Aimee A. Keller, and M. Elizabeth Clarke, and Kathryn Kegel
- NOAA Deep-Sea Coral Research and Technology Program
- National Research Council Research Associate Program
- Crews for the West Coast Groundfish Bottom Trawl Survey, the NOAA RACE Survey program, and MBARI
- Ocean Exploration Trust





# Questions



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Image Credit: DSC RTP